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Serum level of Adrenomedullin in patients with primary knee osteoarthritis; relation to disease severity

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Abstract

Background: Adrenomedullin (AM) is a peptide which was suggested to be involved in the pathogenesis of osteoarthritis through its anti-inflammatory and anti-apoptotic effect. AM was found to be elevated in some inflammatory rheumatic diseases as rheumatoid arthritis and ankylosing spondylitis.

The current study was performed to measure serum Adrenomodullin (AM) concentrations in patients with primary knee osteoarthritis (KOA) and to assess association with severity of the disease. The study was performed on 50 patients with primary KOA diagnosed according to American College of Rheumatology (ACR) Revised Criteria for Early Diagnosis of Knee Osteoarthritis and 20 age- and sex-matched controls with no clinical features of KOA. The Kellgren and Lawrence (KL) classification was used to evaluate the disease severity of knee OA. Disease activity was assessed by The Western Ontario and McMaster Universities Arthritis Index (WOMAC). Blood samples had been collected from patients with OA and controls for assessing Adrenomodullin in patients' sera by ELISA.

Results: There were a significant increase in serum Adrenomedullin concentrations in KOA patients compared to controls (10.64 ±19.2 ng/ml vs. 1.39 ±1.6 ng/ml in cases and controls respectively) (p value = 0.036). There was positive significant correlation of serum Adrenomedullin levels with KL grades (r=0.608, p value <0.001). OA patients with VAS score >6 have significantly higher serum Adrenomedullin levels than OA patients with VAS Score <6. No detected significant correlation between any of (patients' age, BMI, disease duration, tenderness score, and WOMAC score) with serum Adrenomedullin levels among studied OA cases (p values >0.05).

Conclusion: This study concluded that serum Adrenomedullin (AM) level is elevated in patients with KOA and is positively correlated with the severity of disease.

Keywords: Knee osteoarthritis (KOA), Adrenomedullin (AM), WOMAC, KL score

Background

Osteoarthritis (OA) can be regarded as the most prevalent arthritis form. It causes disability in movement that can be defined as patient's need to help for walking or climbing stairs. For patients with affected knees, this disability is more than any other medical condition in the age of 65 [1].

Osteoarthritis is a chronic degenerative joint disease characterized by degraded articular cartilage, damaged subchondral bone, and synovitis. Osteoarthritis causes a significant health problem on communities because it causes limitation in functional activities [2].

Inflammation is considered as a clear pathogenic mechanism for osteoarthritis development and progression that the disease can be regarded as an inflammatory one [3]. Chondrocytes can yield a diversity or inflammatory cytokines. Inflammation has an important role in joints and cartilage loss [4].

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Adrenomedullin is a peptide; its structure includes 52 amino acids and it belongs to the calcitonin/calcitonin gene-related peptide (CGRP) family [5]. It was first recognized in pheochromocytoma of human [6]. Adrenomedullin expression occurs not only in cardiovascular system but also in other tissues as bones and joints. In addition to its strong effect in vasodilation and hypotension, Adrenomedullin also has a role as an anti-inflammatory. Adrenomedullin can effectively suppress the activated macrophages and prevent their production of tumor necrosis factor-α (TNF-α) [7]. Neuropeptides as AM may show strong anti-inflammatory effects. They may control various serious levels of innate immunity [8]. The ability of these neuropeptides to control adaptive immunity has been documented. They can affect activation/differentiation of Th1 cells [9].

Adrenomedullin was found to inhibit interleukin-1-induced proliferation of fibroblast-like synoviocytes which contribute to the development of OA [3]. Cytokines were significantly decreased in the joints of mice after treatment with Adrenomedullin [8].

Adrenomedullin was able to eliminate inflammation and lessen systemic loss of bone in collagen induced arthritis in murines. It can be involved in pathogenesis of osteoarthritis through its anti-inflammatory effect [10].

The current study was designed to assess serum Adrenomodullin concentrations in patients with knee osteoarthritis (OA) and to detect the association with the severity of the disease.

Methods
Patients
This present study was a case-control study conducted on fifty patients (16 males and 34 females) with primary OA of the knee who were diagnosed according to American College of Rheumatology (ACR) Revised Criteria for Early Diagnosis of Knee Osteoarthritis [11], in addition to twenty healthy control subjects, with no clinical features of KOA, matched in age and sex (3 males and 17 females). Patients with generalized erosive OA, other rheumatological, neurological disease, or cancer were excluded (Fig. 1).

Methods
All patients were subjected to full history taking, clinical examination, and routine laboratory investigations. X-ray knee anteroposterior view on standing in osteoarthritis patients. Blood samples had been collected from patients with OA and healthy controls and were kept for assessing Adrenomodullin in their sera.

The Kellgren and Lawrence classification [12] was used to evaluate the disease severity of knee OA. If either knee was assessed as KL grade > and = 2, an OA
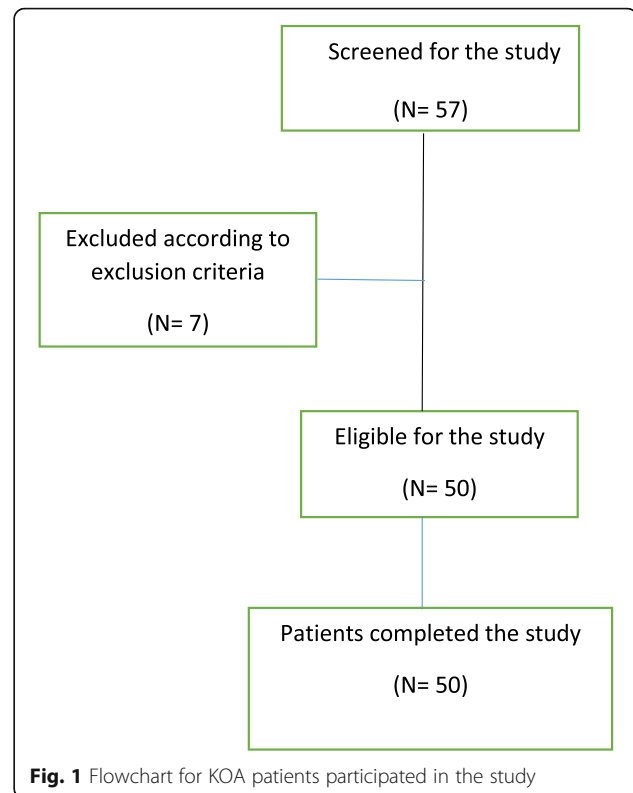

Fig. 1 Flowchart for KOA patients participated in the study

diagnosis was made. Controls were defined as both knee assessed as KL grades of 0.

Assessment of OA disease activity was done using the Western Ontario and McMaster Universities Arthritis Index (WOMAC).WOMAC is a self-administered questionnaire consisting of 24 items divided into 3 subscales: pain (5 items), stiffness (2 items), and physical function (17 items). The test questions are scored on a scale of 0-4, which correspond to the following: none (0), mild (1), moderate (2), severe (3), and extreme (4). The scores for each subscale are summed up, with a possible score range of 0-20 for pain, 0-8 for stiffness, and 0-68 for physical function. A sum of the scores for all 3 subscales gives a total WOMAC score [13].

Assessment of pain using visual analog scale (VAS) is a uni-dimensional measure of pain intensity [14].

Concentrations of Adrenomodullin in serum were measured by ELISA technique using commercially available kits according to the manufacturer's instructions (ELISA kit, Cat No EIA-3418, Lot: 601777, DRG International Inc., USA).

Statistical analysis
The collected data were coded then entered and analyzed using the Statistical Package for Social Sciences (SPSS) computer software (version 25), IBM software, USA. Descriptive statistics was done for categorical variables by frequency and percentage, and for numerical

variables in the form of mean and standard deviation (mean ± SD).

Suitable statistical tests of significance were used (independent sample t test for two unrelated samples, one way ANOVA test for more than two related samples, and post-hoc (LSD) analysis) to identify which pairs of means were statistically different, chi-square ($\chi 2$) test for categorical data, Pearson correlation analysis; and ROC (receiver operator characteristic) curve was used to find out the best cut-off value and validity of certain variable. P values equal to or less than 0.05 were considered statistically significant.

Results

The present case-control study was performed on two matched groups; fifty knee OA patients (case group) distributed as (16) males and (34) females, their ages ranged from 20 to 60 years with a mean of 52.42 ±8.76 years with disease duration ranged from 1 to 20 years with a mean of 6.32 ±4.7 years duration, and 20 healthy individuals (control group) distributed as (3) males and (17) females, their ages ranged from 30 to 60 years with a mean of 50.85 ±9.1 years. Body mass index (BMI) was significantly higher among studied OA cases as compared with healthy control individuals (33.16 ±5.60 vs. 28.53 ±3.71) in OA and healthy controls respectively (p value= 0.001 (Table 1).

All the studied OA cases expressed that they suffer from pain, most studied OA cases expressed suffering from morning stiffness (46 cases out of 50) 92%, while only half of the OA cases suffered from limping (26 cases out of 50), as reported by the patients. Our patients were on non-steroidal anti-inflammatory drugs on demand and were on chondroprotectives irregularly.

Disease activity as assessed by total WOMAC score in OA patients ranged from 40 to 89 with a mean disease activity score of 65.58 ±13.9. Pain intensity assessment by visual analog scale (VAS) score showed that more than half the studied cases [31] had VAS score >6 (62%) while 19 cases had VAS score <6 (38%) (Table 1). As regarding KL score, twenty-three cases were grade 2, fifteen cases were grade 3, and twelve cases were grade 4 with no statistically significant differences between right and left side.

There was a statistically significant higher serum Adrenomedullin levels in the KOA patients compared to the control group (10.64 ±19.2 vs. 1.39 ±1.6) in cases and controls respectively (p value = 0.036) (Fig. 2)

There were no detected significant difference in serum Adrenomedullin levels among studied OA cases according to their sex (p value >0.05).

Spearman correlation analysis showed a positive significant correlation of serum Adrenomedullin levels with

Table 1 Socio-demographic data and clinical assessment of the studied population (N= 70)

	Knee OA patients N= 50	Healthy controls N= 20	p value
Sex; N (%)			
Male	16 (32.0)	3 (15.0)	0.234
Female	34 (68.0)	17 (85.0)	
Age; (years)			
Mean ±SD	52.42 ±8.76	50.85 ±9.1	0.238
Range	20-60	30-60	
BMI; (kg/m^2)			
Mean ±SD	33.16 ±5.60	28.53 ±3.71	**0.001***
Range	22.50-45.00	21.80-33.60	
Disease duration; (years)			
Mean ±SD	6.32 ±4.7	NA	
Range	1.00-20.00		
WOMAC score			
Mean ±SD	65.58 ±13.9	NA	
Range	(40-89)		
VAS; N (%)			
<6	19 (38.00%)	NA	
>6	31 (62.00%)		

OA osteoarthritis, *NA* unrelated, *BMI* body mass index, *WOMAC* Western Ontario and McMaster Universities Arthritis Index, *VAS* visual analog scale
Comparison between cases and controls was done using chi-square test, unpaired t test, or Mann-Whitney U test, as appropriate

KL grades (r=0.608, p value <0.001) in the OA patients (Fig. 3).

No detected significant correlation between any of patients' age, BMI, disease duration, and disease activity as assessed by WOMAC score with serum Adrenomedullin levels among studied OA cases (p values >0.05) (Table 2).

There were significantly higher serum Adrenomedullin levels in the OA case group with VAS score >6 as compared to the OA case group with VAS score <6 (14.60 ± 23.5 vs. 4.18 ±4.2) in VAS score (>6) and (<6) respectively (p value = 0.022).

As demonstrated in Table 3 and Fig. 4, receiver operating characteristic (ROC) curve analysis was used to assess the clinical diagnostic accuracy of serum AM levels in the studied knee OA patients and the normal individuals; the results of serum AM levels (ROC) curve analysis showed p value <0.05, so the serum AM level diagnosed the disease state at a statistically significant level with a 72% sensitivity (true positive cases) and 65% specificity (true negative cases) at a cutoff point level ≥ 2.100.

Discussion

Adrenomedullin is a truncated peptide with antiapoptotic and immunoregulatory properties. It can act as an
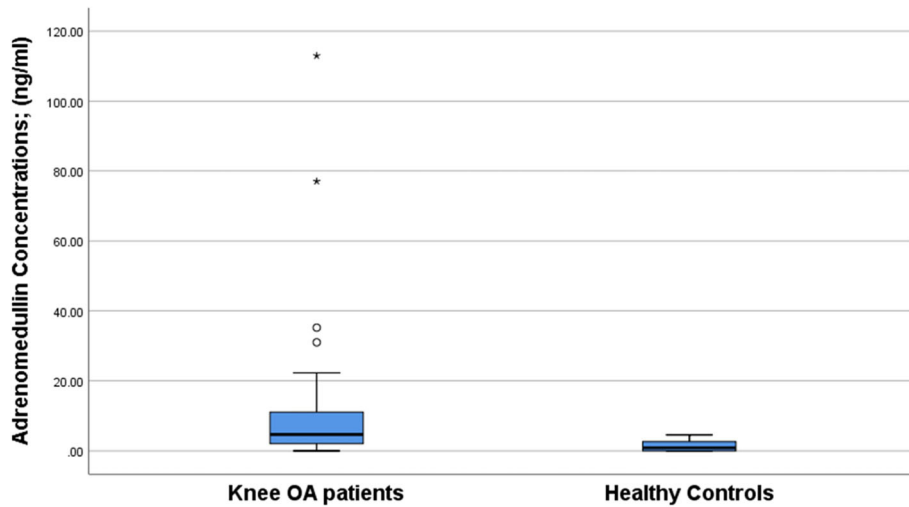
Fig. 2 Serum Adrenomedullin concentrations in the case and control group

agonist or an antagonist depending on cell type. Its in vivo effects are unknown, but Adrenomedullin could possess immunomodulatory properties [15].

Treatment with Adrenomedullin resulted in considerably fewer apoptotic chondrocytes and diminished cartilage degradation in a mouse model of arthritis. After the injection of Adrenomedullin into the knee joint spaces, rabbits with antigen-induced arthritis showed decreased joint swelling [8].

Cytokine expression was studied in mouse joint tissue and serum. In mice with collagen induced arthritis, Adrenomedullin reduced clinical and histologic arthritis scores and shifted the pattern of articular and systemic cytokine expression from Th1 to Th2. Tumor necrosis factor α, interleukin-6 (IL-6), and IL-17A levels were significantly decreased in the joints of mice treated with Adrenomedullin, whereas IL-4 and IL-10 levels were increased [15].

Inflammation is involved in the mechanism of OA. Injection with Adrenomedullin in knee joint spaces of rabbits reduced edematous changes and infiltration of inflammatory cells in the synovial tissues. In addition, Adrenomedullin significantly reduced the expression levels of TNF-α, IL-6, and IL-17A levels [8].

This study was performed to assess of serum Adrenomodullin (AM) concentrations in patients with knee osteoarthritis (OA) and detect correlation with severity of the disease. Majority of our studied OA patients were
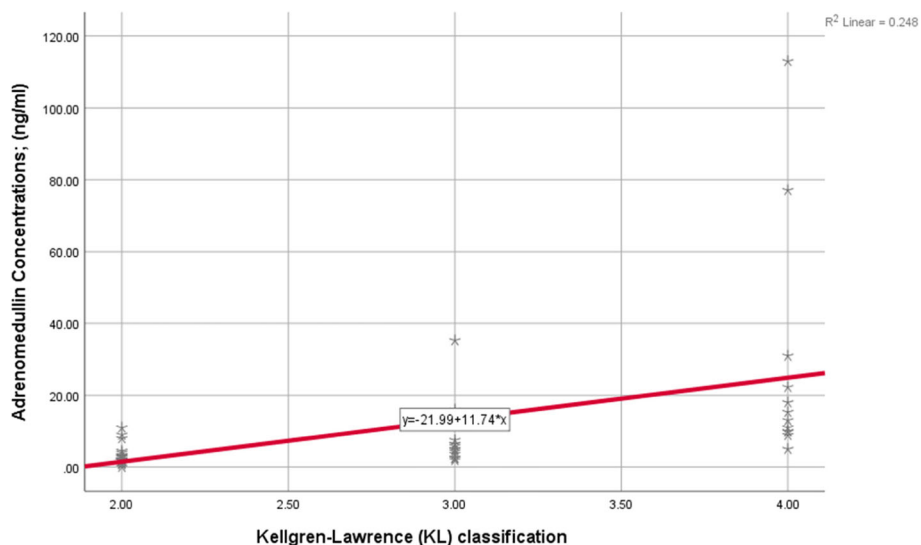

Fig. 3 Correlation between the Kellgren-Lawrence (KL) classification and serum Adrenomedullin concentrations among studied OA cases

Table 2 Spearman correlation between serum Adrenomedullin concentrations and other parameters of the studied OA cases (*N*=50)

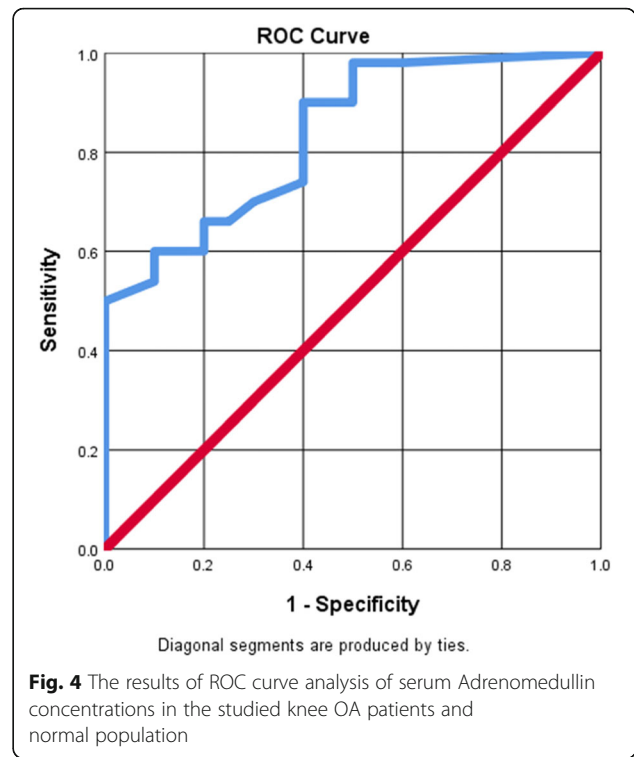|	Adrenomedullin concentrations (ng/ml)	
	r	*p* value
Disease duration (years)	−0.035	0.812
WOMAC score	0.217	0.130
Age (years)	−0.105	0.466
BMI	0.086	0.554

females (68%) with a mean age of 52.42 years; this comes in agreement with the mentioned in literature that OA incidence increases with age and commonly affects females more than males [16].

Although age is intensely related to the development of knee osteoarthritis, overweight is possibly the most significant adjustable risk factor [17]. In harmony with other studies, a strong relation was found in the present study between BMI and knee osteoarthritis, BMI was significantly higher among studied OA cases as compared with healthy control individuals.

In the current study, there was no detected significant correlation between patients age, duration of OA disease with serum Adrenomedullin concentrations in the OA cases.

Body mass index (BMI) has been linked to the prevalence and deterioration of knee osteoarthritis, regardless of age and sex [18]. Even a moderate elevation in BMI was revealed to be significantly associated with knee osteoarthritis [19]. The mechanisms by which obesity is related to the development of knee osteoarthritis are not fully explained. Biomechanical issues (e.g., decreased physical functions and impaired activities, abnormal knee adductor movement, extraordinary burden on the articular cartilage) and metabolic mechanisms (e.g., hormonal dysregulation, liptin) have been proposed as potential facilitating influences for joint osteoarthritis [20].

Higher serum Adrenomedullin levels in the OA group than the control group were detected in the current study with a statistically significant difference. Our findings were in harmony with the reported in similar study implemented in 187 knee OA patients (case group) and 109 healthy subjects (control group) to determine whether serum Adrenomedullin levels were associated with the occurrence and degree of knee osteoarthritis probably as a response to inflammatory cascade; where they reported higher serum

Fig. 4 The results of ROC curve analysis of serum Adrenomedullin concentrations in the studied knee OA patients and normal population

Adrenomedullin levels in the OA group than the control group (*P*<0.001) [21].

The Kellgren and Lawrence (KL) classification was used to evaluate the disease severity and damage of knee OA. Twenty-three cases were grade 2, fifteen cases were grade 3, and twelve cases were grade 4 with no statistically significant differences between right and left side. A positive correlation of serum Adrenomedullin concentrations with KL grades was detected in the present study. This correlation is similar to that reported by Liu and his team in their study; they detected a positive correlation of serum Adrenomedullin concentration with KL grades (*r*=0.322, *P*<0.001) [21].

This increase of serum AM level with the increased severity of OA could be explained by the fact that it is secreted in response to inflammation and increased secretion occur with the degree of inflammation.

Serum Adrenomedullin in this study was higher in the OA case group with VAS score >6 than the OA case group with VAS score <6. It had been found that VAS pain score correlated with Kellgren and Lawrence (KL) classification of disease severity among knee OA [22]; this may explain the statistically significant difference of

Table 3 The results of ROC curve analysis of serum Adrenomedullin concentrations in the studied knee OA patients and normal population

	AUC	SE	95% CI	Sensitivity	Specificity	Cutoff value	*p* value
Adrenomedullin	0.835	0.050	0.737-0.933	72%	65%	≥ 2.100	<0.001

AUC area under the curve, *SE* standard error, *CI* confidence interval of AUC

serum Adrenomedullin concentrations in the OA patients in relation to the VAS score.

The results of our study point to the probability of AM participation in the pathophysiology of joint injuries in patients with OA probably by a combined pro-inflammatory and anti-inflammatory effect. In addition to the fact that AM is recognized to decrease the excretion of cytokines from several cells [23], these results confirm the hypothesis that production of AM in joints and its elevation in the plasma are significantly associated with anti-inflammation in arthritis. Clementi et al. investigated the anti-inflammatory effect of AM in rats, finding that AM production in several cell lines was strongly induced by stimulation of a group of inflammatory cytokines including interleukin 1 and tumor necrosis factor-α [24]. Some studies stated that AM functioned as a circulating vasoactive hormone in blood and plays an anti-inflammatory role in the inhibition of local infection and inflammation, thus playing an important role as a host defense system [25]. Cytokines were significantly decreased in the joints of mice after treatment with Adrenomedullin [8].

Adrenomedullin was found to be elevated in other rheumatic diseases as systemic lupus erythematosus (SLE). El-Srogy et al. detected elevation in serum AM in SLE especially in lupus nephritis patients. AM serum levels were correlated with disease activity in SLE patients. It was negatively correlated with 24 h urine protein excretion in the group of lupus nephritis patients. Serum AM could be considered a biological marker in SLE [26].

In the current study, the serum AM level diagnosed the disease state at a statistically significant level with a 72% sensitivity (true positive cases) and 65% specificity (true negative cases) at a cutoff point level ≥ 2.100. Unfortunately, we were not able to find similar studies that calculated the sensitivity and specificity of AM in the diagnosis of knee OA to compare them with the results of the current study. However, these results may imply that the serum AM level may be an independent biomarker for identification of severity of knee OA among patients.

The current study has its limitations presented as the small sample size and that patients and control are not matched as regard BMI. Also, we did not measure AM level in synovial fluid for financial reasons. Future studies are needed to determine serum and synovial fluid AM to determine if the AM is locally produced in the OA joints and if it is related to synovitis and effusion in order to discuss AM potential use for treatment of OA.

Conclusion
Our results propose that plasma AM levels increase with the severity of OA and could be used as a biomarker to detect degree of disease severity. It might have a role in the treatment of OA due to its anti-inflammatory effects, and its plasma levels could be used as a marker of the degree of OA disease. Our findings need to be supported by additional longitudinal studies with larger sample sizes to detect AM concentrations in both serum and synovial fluid in patients with KOA.

Declarations

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